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SPECIES OF PERENNIAL GRASSES AS FEEDSTOCK FOR BIOGAS PRODUCTION IN THE REPUBLIC OF MOLDOVA

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Abstract

The increasing world population, growing energy consumption, greenhouse gas emission and diminishing supplies of fossil fuels obligate to look for alternative ways, development of new technological processes of energy production. The selection of the appropriate plant species as feedstock for biogas production is an important aspect in decision-making. We investigated some agro-biological peculiarities and biochemical composition of biomass of native perennial grasses Dactylis glomerata, Festuca arundinacea and introduced Agropyron desertorum, Agropyron sibiricum, Miscanthus giganteus, Miscanthus sinensis cultivated on the experimental land of the Botanical Garden (Institute) of the Academy of Sciences of Moldova. The studied species differ in the pace of growth and development, productivity and chemical composition of the harvested mass. The gas forming potential of the fermentable organic matter of biomass varied from 328 to 479 liter/kg volatile solids. The best results were achieved for Dactylis glomerata and Miscanthus sinensis with methane production yield of 248-252 liter/kg volatile solids, likely higher content of nitrogen-free extract. The calculated methane yield per ha for native perennial grasses ranged from 2077 to 2243 m^3/ha, and from 1386 to 1605 m^3/ha – Agropyron species, while from 3348 to 4128 m^3/ha for Miscanthus sinensis and Miscanthus giganteus.

Key words*: agro-biological peculiarities, biochemical composition, perennial grasses, specific methane yield.*

The increasing world population, growing energy consumption, greenhouse gas emission and diminishing supplies of fossil fuels obligate us to look for alternative ways, development of new technological processes of energy production. Using biomass to provide energy services is one of the most versatile options for increasing the proportion of renewable energy in the global energy system.

The biomass resource can be considered as organic matter, in which the energy of sunlight is stored in chemical bonds. The value of a particular type of biomass depends on the chemical and physical properties of the large molecules from which it is made.

Perennial grasses play an important role as an extensive CO_2 sink, significantly increases the content of soil carbon, dry organic matter possesses many beneficial attributes as energy crops, and there has been increasing interest in their use for this purpose in the US and Europe since the mid-1980s (Lewandowski et al., 2003; El Bassam, 2010; Klimiuk et al., 2010).

Miscanthus is a peculiar genus native to East Asia. About 25 species were listed by various researchers. *Miscanthus sinensis* Andersson, *Miscanthus sacchariflorus* (Maxim.) Franch., *Miscanthus floridulus* (Labill.) Warb. ex K. Schum. and Lauterb., *Miscanthus lutarioriparius* L.Liu ex S.L.Chen and Renvoize and the hybrid *Miscanthus giganteus (Miscanthus sinensis* x *Miscanthus sacchariflorus*) Greef et Deu. are mainly used for biomass production because of their high biomass potential (El Bassam, 2010; Arnoult and Brancourt-Hulmel, 2015). At present, China has the largest area under *Miscanthus* cultivation in the world, approximately 100,000 ha, while in Europe, there are estimated 30,000 ha.

Cocksfoot, *Dactylis glomerata* L., and tall fescue, *Festuca arundinacea* Schreb., are native to Europe, differ in their requirements of rainfall, temperature and soil type and fertility for growth, therefore they have a varying degree of adaptation in different regions, are highly productive and can be used as fodder and pasture. These species are investigated in many scientific centres and implemented as crop in different regions of the Earth, not only as a source of fodder, but also as feedstock for bioenergy production (El Bassam, 2010; Tilvikiene et al., 2012). They are also common

in the spontaneous flora of the Republic of Moldova, there have been researched and selected forms that have a productivity of 50-65 tons/ha of fresh mass or 15-17 tons of hay (Bahcivanjii et al., 2012).

Taking into account the expansion of areas of degraded soils and the frequency of droughts, it is necessary to mobilize and introduce new species that would ensure production in these harsh conditions. The most promising perennial grasses to be introduced and that may contribute to the solution of these problems are the species of the genus *Agropyron* Gaertn.: desert wheatgrass, *Agropyron desertorum* (Fischer ex Link) Schultes (syn. *Triticum desertorum* Fischer ex Link) and Siberian wheatgrass *Agropyron sibiricum* (Willd.) Beauv. *Agropyron fragile* (Roth) Candargy (syn. *Agropyron fragile* (Roth) Candargy; *Triticum fragile* Roth; *Triticum sibiricum* Willd), their natural distribution range – from the Central Europe and the Middle East across Central Asia to Siberia, China and Mongolia. They are long-lived, cool-season, drought tolerant, and winter hardy grasses with extensive root systems that may extend past 2 m into the soil, contributing to stabilize soils and reduce erosion. Desert wheatgrass is best suited for loamy and clayey light chestnut soils and solonetz soils. *Agropyron desertorum* and *Agropyron sibiricum* provide highly nutritional forage for livestock, especially in the early spring, furnish hay of excellent quality and remain productive for more than 30 years (Medvedev and Smetannikova, 1981).

The Republic of Moldova imports 95% of fossil energy resources, according to the Energy Strategy of the Republic of Moldova (2013), the total amount of energy produced from renewable sources should be increased to 20% by the year 2030 and ¾ of this amount will make energy from biomass.

In the last 65 years, as a result of the introduction and acclimatization researches done in the Botanical Garden (Institute) of the ASM, collections and exhibitions of plants with multiple uses, necessary for the development of the national economy, were founded. The investigation of local and introduced plant species for biomass production is an important objective (Teleuta et al., 2012; Ţîţei, 2013; 2015).

The selection of the appropriate plant species as feedstock for biogas production is an important aspect in decision-making.

The aim of this work was to evaluate the native species *Dactylis glomerata*, *Festuca arundinacea,* the introduced species *Agropyron desertorum*, *Agropyron sibiricum, Miscanthus sinensis* and hybrid *Miscanthus giganteus* as feedstock for biogas production based on the chemical composition and biomass yield under the conditions of the Republic of Moldova.

MATERIALS AND METHODS

The perennial grasses: the native species *Dactylis glomerata, Festuca arundinacea,* the introduced species *Agropyron desertorum*, *Agropyron sibiricum, Miscanthus sinensis* and the hybrid *Miscanthus giganteus*, maintained in monoculture, served as subject of research. The experiments were performed on non irrigated experimental land in the Botanical Garden (Institute) of the ASM. The experiments started in spring, when the soil had reached the physical readiness, with seeds of *Agropyron desertorum*, *Agropyron sibiricum*, *Dactylis glomerata*, *Festuca arundinacea* and rhizomes of *Miscanthus giganteus* and *Miscanthus sinensis.* The experimental design was a randomised complete block with four replications, and the experimental plots measured 10 m^2. The plant growth, development and productivity were assessed according to the methodical guidelines (Novoselov et al., 1983).

The species of perennial grasses were harvested by hand in different periods and development stages: *Agropyron desertorum*, *Agropyron sibiricum*, *Dactylis glomerata* and *Festuca arundinacea* – in the flowering stage (26.05.2014), but *Miscanthus sinensis* and *Miscanthus giganteus* – in the inflorescence formation period (09.07.2014).

Fresh mass yield was measured by weighing. Dry matter or total solid (TS) content was detected by drying samples up to constant weight at 105°C. Crude protein – by Kjeldahl method; crude fat – by Soxhlet method, crude cellulose – by Van Soest method; ash – in muffle furnace at 550°C (Petukhov et al., 1989). Nitrogen free extract (NFE) was mathematically appreciated, as difference between organic matter values and analytically

assessed organic compounds. Organic dry matter or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter.

Theoretical biogas normalized litre per kg of volatile solids and methane yields were calculated using the gas forming potential of nutrients according to Baserga (1998). The biogas production potential and specific methane yields were evaluated by the parameter "content of fermentable organic matter", according to Weissbach (2009).

RESULTS AND DISCUSSIONS

In the first year of vegetation, the studied species of perennial grasses were characterized by a different pace of growth and development. It was found that the emergence of seedlings of the species *Agropyron desertorum*, *Agropyron sibiricum*, *Dactylis glomerata* and *Festuca arundinacea* at the soil surface took place simultaneously after 10-13 days from sowing. The species *Agropyron desertorum* and *Agropyron sibiricum* developed 48-66 cm tall shoots, went through all ontogenetic stages and formed seeds, but *Dactylis glomerata* and *Festuca arundinacea* did not develop shoots. The species of the genus *Miscanthus* were distinguished by faster growth, they developed shoots that reached a height of 114-128 cm – *Miscanthus sinensis* and 152-183 cm – *Miscanthus giganteus*, and finished their growth cycle during the flowering stage.

We might mention that, in the following years, the regrowing season for the species *Agropyron desertorum*, *Agropyron sibiricum*, *Dactylis glomerata* and *Festuca arundinacea* started in the first half of March, when the average temperature was above 3-5ºC and for *Miscanthus sinensis* and *Miscanthus giganteus* – in April. The resumption of growth of the species *Agropyron sibiricum* took place 8 days earlier in comparison with *Dactylis glomerata* and 39 days – in comparison with *Miscanthus giganteus*. *Dactylis glomerata* was distinguished by a more intense development and required a shorter period for the formation and maturation of seeds. The plants of the genus *Miscanthus*, from the resumption of growth to the formation of inflorescences required a period of 83-95 days and 99-125 days to full flowering, the hybrid *Miscanthus giganteus* developed later (Table 1).

Table 1. Agro-biological peculiarities of the studied species of perennial grasses, 2014

Indicators	*Festuca arundinacea*	*Dactylis glomerata*	*Agropyron sibiricum*	*Agropyron desertorum*	*Miscanthus sinensis*	*Miscanthus giganteus*
Beginning of vegetation	10.03	15.03	7.03	10.03	12.04	15.04
Days up to:						
- inflorescence formation	66	60	66	65	83	95
- flowering	86	72	83	88	99	125
- seed ripening	122	111	127	118	126	-
Plant height, cm						
- on April 20	76	72	62	59	12	8
- at flowering	131	112	100	93	188	324
The yield, 1st harvest						
- fresh mass, kg/m^2	4.08	3.89	2.05	2.43	4.22	7.54
- dry matter, kg/m^2	0.88	0.89	0.70	0.66	1.35	2.40

The studied species differed also in the pace of growth. Thus, by late April, *Dactylis glomerata* and *Festuca arundinacea* plants reached a height of 72-76 cm, the plants of the genus *Agropyron* – 59-62 cm, and the plants of the genus *Miscanthus* – 8-12 cm, and by full flowering – 112-131 cm, 93-100 cm and 188-324 cm, respectively.

It is known that the pace of growth and development of plants influences the biomass accumulation and the dry matter content. Among the species of perennial grasses that were harvested in the end of May, the native species *Dactylis glomerata* and *Festuca arundinacea* produced the highest yield of fresh mass – 3.89-4.08 kg/m^2, but the introduced species of the genus *Agropyron* – 2.05-2.43 kg/m^2. The species *Agropyron sibiricum* is characterized by high content of dry matter in the harvested biomass.

In Lithuania the variation of dry matter yield of tall fescue ranged from 13.37 to 13.84 t/ ha, of cocksfoot from 10.39 to 11.47 t/ha (Tilvikiene et al., 2012). Romanian varieties *Dactylis glomerata* and *Festuca arundinacea* had a productivity of 55-75 t/ha fresh mass or 12-17

t/ha dry matter (Maruşca et al., 2011). In Russia hay productivity of *Agropyron* species varies from 2.2 to 4.0 t/ha (Medvedev and Smetannikova, 1981).

The species of the genus *Miscanthus* grew faster in late May. At harvest time, the fresh mass yield and the dry matter content of these species was higher. So, the yield of *Miscanthus sinensis* harvested in the first decade of July reached 4.22 kg/m^2 fresh mass or 1.35 kg/m^2 dry matter, and *Miscanthus giganteus* – 7.54 kg/m^2 fresh mass or 2.40 kg/m^2 dry matter.

Organic dry matter or volatile solid yield is an important factor influencing biogas and methane yield. The studied species differ in the content of volatile substances from 911.1 to 951.8 g/kg. A lower content was found in *Festuca arundinacea, Dactylis glomerata* and *Miscanthus giganteus,* and the highest one – in *Miscanthus sinensis.* In the harvested biomass of the species of the genus *Agropyron*, the content of volatile substances was high.

Crude protein is the main nitrogen-containing nutrition component for microbes converting biomass. Analyzing the obtained data, we could mention that the local species *Dactylis glomerata* and *Festuca arundinacea* were characterized by a high content of protein (7.42-9.51% TS) in comparison with the species of the genus *Miscanthus* (5.41-5.63% TS). The introduced species *Agropyron desertorum* and *Agropyron sibiricum* didn't differ essentially in the content of crude protein (Table 2).

It is well known that fat is a good source of energy (Baserga, 1998; Weissbach, 2009). A higher content of fat was found in the species *Festuca arundinacea* and *Agropyron sibiricum*, the species *Dactylis glomerata* and *Miscanthus sinensis* were at the same level, but *Agropyron desertorum* and the hybrid *Miscanthus giganteus* had a low content of fat.

Carbohydrates supply most of the energy for maintaining vitality. The two carbohydrate fractions commonly used in evaluating the carbohydrate content of feed are crude cellulose and nitrogen-free extract.

It was found that the studied species had a high content of crude cellulose, which varied from 35.59% to 45.28%. The harvested biomass of *Miscanthus giganteus* and *Agropyron sibiricum* was characterised by a very high content of crude cellulose – 41.60-45.28%, and the local species *Festuca arundinacea* and *Dactylis glomerata* had a moderate content of 35.59-37.29 %. Nitrogen-free extract comprises sugars, starch and a large part of the material classed as hemicellulose. In the biomass of the investigated plant species, the nitrogen-free extract varied from 41.45% to 49.76%. Lower nitrogen-free extract content was noted in the biomass of the species *Festuca arundinacea* and the hybrid *Miscanthus giganteus*, higher content – in *Miscanthus sinensis* (49.65), *Dactylis glomerata* (46.62%).

The theoretical biogas yield ranged from 722 to 756 l/kg VS (Table 3). The calculated methane content in the biogas ranged from 50.8 to 53.9 %. The native species *Dactylis glomerata* and *Festuca arundinacea* had a reduced potential of biogas (722-726 l/kg VS), but were distinguished by a higher content of methane (52.0-53.9%).

The theoretical methane yield ranged from 2541 to 9192 m^3/ha and it depended on the biomass productivity of the studied species. The species *Agropyron desertorum* and *Agropyron sibiricum* were less productive: 2541-2737 m^3/ha, and the species of the genus *Miscanthus* reached values of 5184-9192 m^3/ha, the hybrid *Miscanthus giganteus* reached the highest values.

Differences in gas formation potentials of crops are mainly due to specific chemical compositions of the plant material.

Table 2. Biochemical composition of the biomass of the studied species of perennial grasses, 2014

Indicators	*Festuca arundinacea*	*Dactylis glomerata*	*Agropyron sibiricum*	*Agropyron desertorum*	*Miscanthus sinensis*	*Miscanthus giganteus*
Dry matter contains:						
crude protein, *%*	9.51	7.42	7.01	6.85	5.63	5.41
crude fat, *%*	2.86	1.96	2.62	1.58	1.96	1.62
crude cellulose, *%*	37.29	35.59	38.51	41.60	37.83	45.28
nitrogen free extract, *%*	41.45	46.62	46.12	44.05	49.76	41.52
crude ash,*%*	8.89	8.41	5.74	5.92	4.82	6.17
organic dry matter, *%*	91.11	91.59	94.26	94.08	95.18	93.83

Table 3. Stoichiometric gas production potential of the biomass of the studied species of perennial grasses, 2014

Indicators	*Festuca arundinacea*	*Dactylis glomerata*	*Agropyron sibiricum*	*Agropyron desertorum*	*Miscanthus sinensis*	*Miscanthus giganteus*
Biogas, liter /kg VS	722	726	750	744	756	744
Biomethane, liter /kg VS	382	378	391	385	384	383
Methane content %	53.9	52.0	52.1	51.8	50.8	51.5
Theoretical methane yield, m^3/ ha	3662	3364	2737	2541	5184	9192

Table 4. Gas forming potential of the fermentable organic matter of the studied species of perennial grasses, 2014

Indicators	*Festuca arundinacea*	*Dactylis glomerata*	*Agropyron sibiricum*	*Agropyron desertorum*	*Miscanthus sinensis*	*Miscanthus giganteus*
Fermentable organic matter, g/kg VS	561	599	567	499	590	410
Biogas, liter /kg VS	449	479	454	399	472	328
Specific methane yield, liter /kg VS	236	252	238	210	248	172
Methane yield, m^3/ha	2077	2243	1666	1386	3348	4128

The capability of biomass methanization is tightly associated with nutrient digestibility and plant species. When crude cellulose content increases, digestibility usually decreases. Nitrogen-free extract contains the most digestible portion of the carbohydrates. The research performed by many authors proved that high lignin content significantly reduces the digestibility of plant material and effectively inhibits the process of anaerobic digestion (Dandikas et al., 2015). Fermentable organic matter represents the proportion of organic matter which can be biologically degraded under anaerobic conditions and, thus, can be potentially utilized in biogas facilities (Weissbach, 2009). Gas forming potential of the fermentable organic matter of biomass of the studied species of perennial grasses varied from 328 to 479 l/kg VS (Table 4). The best results were achieved for *Dactylis glomerata* and *Miscanthus sinensis* with methane production yield of 248-252 l/kg VS, likely higher content of nitrogen-free extract. The lowest methane potentials per VS were found in the biomass of *Miscanthus giganteus* and *Agropyron desertorum.* The obtained values are in good accordance with Lemmer and Oechsner (2001) who observed similar values for fresh grass of 0.23-0.41 m^3/kg VS and Amon et al. (2004) who observed 0.25 m^3/kg VS, methane yields from grasses with intensive growth after the early harvest at mid May were larger with 310-360 l/kg VS (Mähnert et al., 2002), *Miscanthus giganteus* (silages), harvested in autumn, reached values of 0.10 m^3/ kg VS (Klimiuk et al., 2010).

The methane yield per ha for native perennial grasses ranged from 2077 to 2243 m^3/ha, and from 1386 to 1605 m^3/ha – *Agropyron* species, while from 3348 to 4128 m^3/ha for *Miscanthus sinensis* and *Miscanthus giganteus*, respectively.

The research showed, however, that the degree of organic conversion into methane, respective methane yields of theoretical yield, was 45% for *Miscanthus giganteus* and 65% for *Miscanthus sinensis*, 61% for *Dactylis glomerata* and *Agropyron desertorum,* 55-57% for *Agropyron sibiricum* and *Festuca arundinacea.*

CONCLUSIONS

The studied perennial grasses species differ in the pace of growth and development, productivity and chemical composition of the harvested mass, which have influenced the methane yield. The gas forming potential of the fermentable organic matter of biomass varied from 328 to 479 l/kg VS. The best results were achieved for *Dactylis glomerata* and *Miscanthus sinensis* with methane production yield of 248-252 l/kg VS, likely higher content of nitrogen-free extract. The calculated methane yield per ha for native perennial grasses ranged from 2077 to 2243 m^3/ha, and from 1386 to 1605 m^3/ha – *Agropyron* species, while from 3348 to 4128 m^3/ha for *Miscanthus sinensis* and *Miscanthus giganteus*, respectively.

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